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RESEARCH ARTICLE

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Analysis of Pesticides in Pollen using LC-MS and QuEChERS Techniques

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ABSTRACT
A comparative study was conducted between the conventional QuEChERS method for the determination of pesticide residues in pollen and a modified method, in order to select the method that works properly. To do this, few pesticides were analyzed using LC-MS (liquid chromatography coupled to mass spectrometry); the comparison of the methods was made using different validation parameters such as: recovery, precision (such as repeatability) and detection limits, which were estimated by different approaches. In addition to this, a factorial experimental design was carried out that allowed evaluating the efficiency of both methods in different types of pollen. The results indicated that the method developed is suitable for the analysis of pesticide residues in pollen. Likewise, it was found that the developed method has better detection limits, better accuracy and better selectivity than the conventional QuEChERS method for the analysis of pesticides under study in the bee pollen matrix. Finally, it was found that the modified method is not suitable for the analysis of three of the 26 pesticides studied.

Keywords: Pesticides, QuEChERS, Pollen, Validation.

Introduction

The use of chemical products for the protection of hives and the presence of pollutants in the environment such as heavy metals, pesticides, antibiotics, among others, creates an imminent threat in the commercialization of bee products, mainly due to the adverse effects of these substances on the health of consumers (1-3). Therefore, most of the countries or communities that consume these products, such as the United States, Canada, Japan or the European Union, have established strict regulations for the marketing of this type of food, so that the presence of these substances or elements , it can become a phytosanitary barrier when exporting bee products to these countries (4-6). These laws have led the scientific community to produce an important analytical development that allows the detection and quantification of this type of contaminants, in order to control the safety and quality of this product (7-9). The developed methodologies must have minimum requirements, such as having quantification limits (LC) low enough to determine the demanding maximum residue limits (MRLs) imposed for the different pollutants. Additionally, in the case of

pesticide and antibiotic residues, it is necessary that these methodologies have sufficiently selective and sensitive technology to avoid both false positives and false negatives (7). That is why, during the last decade, development and research have increased in everything related to methods of detecting contaminants in hive products, especially chromatographic techniques coupled to mass spectrometry (8, 10).

At present, it is recognized that the QuEChERS method is undoubtedly one of the methods of greater robustness, simplicity, reproducibility, versatility, among other benefits (11). The original method was developed for the analysis of pesticide residues in fruits and vegetable matrices, therefore, in recent years different variations have been developed in order to adapt the method to specific matrices or analytes, such is the case of analysis of pesticides, antibiotics, phenols or other contaminants in matrices such as milks (12), soils (13), cereals (14), oils (15), among others (16, 17).

For the specific case of bee products, and especially when working with honey, it is found that the original method was modified with the purpose of eliminating interference (18). In this way, it was decided to take a smaller amount of sample (1 to 5 times less than the original method), this modification implies that there is the latest technology such as gas chromatographs or liquid chromatographs coupled to spectrometry equipment masses with flight time analyzers, orbitrap, triple quadruple, linear ion trap, among others, mainly due to the selectivity and sensitivity required (18, 19). The analysis of pesticide residues in apicultural products is well known for its usefulness as an environmental indicator, however there are few works that perform pollen analysis (9, 20, 21); This situation is possibly due to the complexity of this matrix and to the fact that the majority of monitoring results have been carried out through chromatographic techniques coupled to tandem mass spectrometers, which do not require modifications of the QuEChERS method. The existing publications, within our knowledge, use another type of methods (20) or modify the sample quantity to approximately one third (22), which has allowed us to obtain successful results, however none of the publications has reported more than 30 pesticides using the same instrument.

In this context, the present work aims to compare a modified version of QuEChERS with the original method, with the purpose of evaluating whether the modifications made allow to extend the scope of the method. In addition, it is intended to evaluate the effectiveness of the modified method in the recovery of pesticide residues in different types of pollen.

Chromatographic conditions

The chromatographic analysis was carried out on an Ultra-Fast Liquid Chromatograph (UFLC) Shimadzu Prominence, coupled to a selective mass detector LCMS-2020.

The analyzes were carried out on a Shim Pack column (6 cm × 2 mm ID, particle size of 2.1 µm and stationary phase C18), worked in gradient mode with 0.1% formic acid (p / v) and 5 mM ammonium acetate in Milli-Q water (A), the organic phase used was acetonitrile (B). The elution program used expressed as a percentage of B, starts at 0% (0 min) increases to 20% in 0.01 min, then reaches up to 25% at 0.30 min and then increases to 100% in the following 10 min, finally held for 0.20 min. To restore the mobile phase to the initial condition of the analysis, it goes from 100% to 0% of B in 2 min where it is maintained for 5 min to balance the column. The data was acquired during the first 8 min. The injection volume was 5 µL, the column temperature 40 ° C and the mobile phase flow 0.3 mL / min.

Interface and mass spectrometer conditions

The equipment has a DUIS type interface (ESI, APCI), which was operated in ESI mode, with a drying gas flow of 10 L / min and a nebulizer gas flow of 1.5 L / min. The temperatures of the heating block and the solvent removal line corresponded to 190 ° C and 250 ° C respectively.

The analyses were carried out simultaneously in positive and negative mode, the voltage applied in the capillary corresponded to 4500 V and -4500 V, respectively. All analyses were performed in selective ion monitoring (SIM) mode. Table 1 shows the ions selected for quantification and retention time of each compound. These compounds were selected taking into account international legislation (5), the level of involvement of bees (3), and their use in India, among other considerations.

Table 1. Quantification lines (T) for the compounds studied and retention times for the two acquisition methods used

METHOD 1			METHOD 2		
Compound	$t_{Retention}$ (min)	Ion T (m/z)	Compound	$t_{Retention}$ (min)	Ion T (m/z)
Acephate	2.56	20	methamidophos	2.54	42
Monocrotophos	2.78	24	propamocarb	2.63	89
Oxamyl	2.91	237	Thiocyclam	2.68	82
Metomil	3.06	63	Linuron	2.70	247
Benomyl	3.15	92	nitenpyram	2.79	27
Imidacloprid	3.52	254	thiamethoxam	2.99	292
Dimetoato	3.63	230	dazomet	3.03	63
tiabendazole	3.24	202	mevinphos	3.17	225
cymoxanil	3.86	97	3-oh carbofuran	3.17	255
thiodicarb	4.83	355	acetamiprid	3.30	223
Carbofuran	4.81	222	thiacloprid	3.71	297
atrazine	5.19	216	dinotefuran	2.70	203
metalaxyl	5.34	280	somazina	4.08	202
Pirimicarb	4.81	239	carbaryl	4.57	219
imazalil	4.66	297	isoprocarb	5.90	235
3,4 -DPA	5.89	216	Ametrina Proficol	6.17	269
pyrimethanil	6.03	200	tridemorph	6.62	298
dimethomorph	5.93	390	epoxiconazole	6.70	330
azoxystrobin	6.49	404	fenhexamid	7.81	300
tebuconazole	6.79	308	ethoprophos	7.81	284
methoxyfenozide	6.86	369	flusilazole	7.94	357
hexaconazole	7.00	358	penconazole	8.07	325
spinosad a	6.33	733	pyraclostrobin	9.80	388
benalaxyl-m	7.73	326	trifloxystrobin	9.17	409
difenoconazole	7.69	408	lufenuron	9.41	509
azinphos methyl	7.78	368	buprofezin	9.91	306
spinosad d	6.49	747	clorfenamina	7.74	349
indoxacarb	8.27	528	temefos	9.66	467

Unmodified QuEChERS method

For the extraction of the pesticides by means of the unmodified QuEChERS method, 10 g of sample were weighed in a centrifuge tube, then TPP (triphenyl phosphate) and 200 µL of a pesticide mixture were added to obtain the concentrations presented in Table 2 the mixture was allowed to stand for 10 min and, after that time, 10 mL of acetonitrile, 4 g of anhydrous $MgSO_4$ and 1 g of AcONa were added and the mixture was stirred for 1 min manually. Subsequently, it was centrifuged at 4500 rpm for 5 min and, with the help of a pipette, 10 mL of the supernatant was taken, which was transferred to a 15 mL centrifuge tube. For the cleaning process, 25 mg of primary / secondary amine (PSA) and 150 mg of anhydrous $MgSO_4$ were added per milliliter of extract, then stirred manually for 30 s and centrifuged for 2 min at 4500 rpm Then 5 mL of the

supernatant was taken and concentrated with nitrogen to dryness, then reconstituted to 1 mL with acetonitrile. Finally, the supernatant was filtered through a 0.22 µm PTFE membrane and transferred to a chromatography vial (27).

Modified QueChERS method

For extraction using the modified QuEChERS method, 10 g of sample was weighed in a centrifuge tube, then TPP and 200 µL of a pesticide mixture were added to obtain the concentrations of Table 2. The resulting solution was allowed to stand by 10 min and, after that time, 10 mL of a citrate buffer solution at a pH of 6.2 was added. The tube was then gently shaken so that the solution came into contact with the pollen. After that, 10 mL of acetonitrile were added, the tube was covered and stirred for about 10 min in a vortex, after this time the tube was taken to an ultrasound bath for 10 min. Next, 4 g of anhydrous MgSO4 were added, stirred vigorously for 1 min and centrifuged for 3 min at 7000 rpm.

With the help of a pipette, 8 mL of the supernatant was taken, which was transferred to a 15 mL centrifuge tube. For the cleaning process, 25 mg of PSA (primary / secondary amine), 25 mg of C18, and 150 mg of anhydrous MgSO4 were added per milliliter of extract, stirred manually for 30 s and centrifuged for 2 min at 4500 rpm . Subsequently, 5 mL of the supernatant was taken and concentrated with nitrogen to dryness, then reconstituted and titrated to 1 mL with a mixture of ACN: Water 7: 3 (v / v). Finally, the sample was filtered through a 0.22 µm PTFE membrane, and transferred to a chromatography vial.

Method Comparison

The comparison of the methods was carried out through the evaluation of different validation parameters, including: (i) detection limits, which were estimated and confirmed based on the IUPAC method and the t_{99} method (27); (ii) selectivity, which was evaluated with 5 different targets of different origin (iii) accuracy, evaluated as a standard deviation relative to three different concentration levels under repeatability conditions. Finally, in order to make a more objective comparison of the pesticides for which both methods were selective and precise, an experimental design with a 2x3 factorial structure was made, in which the recovery of the pesticides under study at two different concentration levels was evaluated (factor 1) and in three different types of pollen (factor 2), which were differentiated by the crop and the region where they came from. For this study, pollen samples free of pesticides were fortified, which was verified by an analysis before fortification.

All statistical analyzes were performed at a 95% confidence level with the SPSS v22 statistical program (2013). The minimum number of replicas for the experiments corresponded to four. Fortification concentrations for the trials are presented in Table 2.

Results and Discussion

Selectivity of the methods

For the QuEChERS method it was found that for diphenoconazole, indoxacarp, buprofezin, espinosad A, benfuracarb, lufenuron, chlorfenaprid, temephos, benalaxil and trifloxtribin, they presented chromatographic signals that prevented the detection of the compounds (interferences), which were superior to the chromatographic response of the analyte at a concentration equivalent to the maximum residue limit (10 µg / kg); which is the concentration that is adopted by default in European legislation in the case where there are no established MRLs for a compound (5). On the other hand, in the case of the modified QuEChERS method, adequate selectivity was found for all the compounds and although some interference occurred in some targets they never exceeded 30% of the value of the chromatographic response for the reference concentration (28) .

According to the selectivity results of the unmodified QuEChERS method, it is evident that there is a relatively high number of compounds that exhibit significant interference; these are equivalent to about 21% of the total compounds analyzed in the present study. The compounds mentioned above have in common that none elute at retention times of less than 6 min (Table 1), which indicates that they are retained in the column and therefore presumed to be lipophilic in nature or have a dipole moment low. The above raises the

possibility of having low polarity interferers that co-elude with the analytes of interest. In this sense, it is understood that the modified method probably presents better selectivity for two reasons. First, the use of octadecylsilane (C18) adsorbents in the cleaning process allows eliminating some of the lipophilic interferences that produce an increase in retention times. On the other hand, the use of graffiti black carbon (GCB) can also contribute to the elimination of this type of interference, because this type of adsorbent is known to reduce the presence of planar compounds, such as chlorophylls and some pigments, which are found in this type of material due to their plant origin (29). The second reason why the selectivity of the method is believed to be improved corresponds to the change of solvent that is made to reconstitute the sample once it is concentrated through a nitrogen flow. During the development of the method, it was observed that upon reconstitution with a mixture of acetonitrile-water (7: 3) it was possible to obtain an extract with a coloration less than that obtained when reconstitution with acetonitrile. The above suggests that some matrix compounds (since none of the pesticides at these concentrations cause this type of coloration), are not dissolved and therefore the extracts have a lower number of interferents in the detection process. On the other hand, the nature of these compounds should be lipophilic, otherwise the coloration would not depend on the polarity of the solvent in which the reconstitution is performed.

Method detection limits

Table 3 presents the results of the confirmation of the estimated detection limits for some selected compounds. The remaining compounds are not presented because the results, between the methods, are similar in magnitude.

The results in Table 3 show that in all cases the detection limits (LD) obtained by the modified QuEChERS method are lower, that is, through this methodology there is a greater detection capacity (lower LD). On the other hand, for compounds such as indoxacarb, spinosad, diphenoconazole and chlorfenaprid that presented selectivity problems using the QuEChERS method, higher detection limits were presented (up to 10 times), with respect to the modified method. Also, for these compounds it is found that through the modified method better signal / noise ratios are obtained, even when lower concentrations of analyte are used.

Obtaining higher detection limits when using the unmodified QuEChERS method is attributed to its low selectivity; which causes a greater noise, a greater standard deviation of the targets and a lower signal / noise ratio. On the other hand, although several compounds such as carbofuran, metalaxyl and hexaconazole did not show selectivity problems through the QuEChERS method, it can be observed that they had a higher detection limit and a lower signal / noise ratio (Table 3), which indicates that there is possibly a greater background noise, which although it does not turn out to be significant (less than 30% of the response of the lowest concentration) is greater than in the case of the modified method. In addition, it was found that by the modified QuEChERS method, none of the compounds presented in the table have signal-to-noise ratios of less than 3, while for the unmodified QuEChERS method several compounds with signal-to-noise ratios of less than 3 are found, which confirms that the detection capacity of the modified method is superior.

Method repeatability

The repeatability of the methods at three different concentrations was evaluated, Table 2 shows the lowest concentration of fortification, which is the concentration that is adopted as the limit of quantification (LC), and the other fortifications were performed at twice the LC and five times the LC.

In order to assess whether the concentration influenced the repeatability of the method, the variance of the residuals was analyzed by the Levene method (30). The results of this test are presented in Table 2. It should be noted that in this test, a probability of less than 0.05 indicates that there are significant differences between the residuals at the different fortification concentrations, that is to say that the accuracy changes as a function of concentration. In this sense, the results shown in Table 2 indicate that most of the compounds do not change their accuracy depending on the concentration, in fact in the QuEChERS method none of the compounds have significant differences (in all cases $p > 0.05$). In the case of the modified method, it is found that for compounds such as simazine, thiabendazole and mevinfos there are changes in the precision of the

method ($p < 0.05$), however, as will be observed later none of these changes exceeds the acceptance criteria of repeatability (28).

Figures 1 and 2 show the maximum coefficients of variation obtained for each of the analytes in the different fortification concentrations. In these figures it is observed that for the majority of cases there are coefficients of variation below 15% (threshold established by the European Union for the analysis of pesticide residues in food (28)), however, in some cases (eg monocrotophos, metomyl, pyrimicarb, indoxacarb, among others) analytes have coefficients of variation above this threshold, that is, the method does not turn out to be precise enough for these compounds. This fact can be attributed to different causes: (i) the increase in the adsorption processes of some analytes at fortification concentrations (see Table 2), (ii) heterogeneity of the extraction mixture (acetonitrile-pollen), (iii) temperature changes at the time of the addition of $MgSO_4$, (iv) stability of the analytes in the pollen and in the extraction mixture, (v) matrix effect at the mass spectrometer interface, (vi) coelution of compounds of the matrix, (vii) low selectivity of the method, among others. Finally, it should be noted that the modified method proved to be precise since variation coefficients of less than 15% were obtained (except for temephos and Benomyl), under repeatability conditions.

Table 2. Recovery percentages obtained in the evaluation of different cleaning systems

Compound Name	Average% recovery			
	PSA	PSA + CNG	PSA + C18	PSA + C18 + CNG
Methamidophos	1	1	87.6	80.2
Propamocarb	1	1	93.5	90.2
Linuron	1	1	100.9	96.8
nitenpyram	1	1	100.3	95.3
Linuron	1	1	97.3	99.2
Nitempiram	1	1	98.2	90.2
3,4 dicloropropinalidina	1	94.4	97.4	92
Ethoprophos	1	1	100.3	98.3
pyraclostrobin	1	94.0	103.4	92.7
Trifloxystrobin	1	I	1	1
lufenuron	1	1	100.1	9 .2
Thiocyclam	1	1	96.8	92.9
Atrazine	1	1	1	1

In Figures 1 and 2 it can be seen that in general for the majority of the compounds better coefficients of variation were obtained using the modified method, this is due to a better homogenization and a better pH control (since the dissolved buffer is added in water and not in solid state). Additionally, another cause that contributes to obtain better coefficients of variation with this method is the use of the vortex, which homogenizes the sample better and more efficiently than the hand. In addition, the use of ultrasound to assist the extraction process improves the mass transfer that occurs from the pollen to the extractant phase.

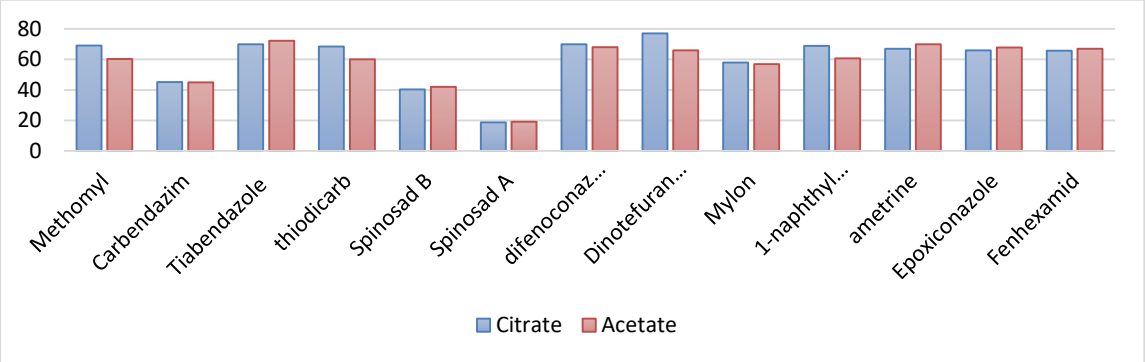

Figure 1: Comparison of recovery percentages to use acetate or citrate as a way out of pH control

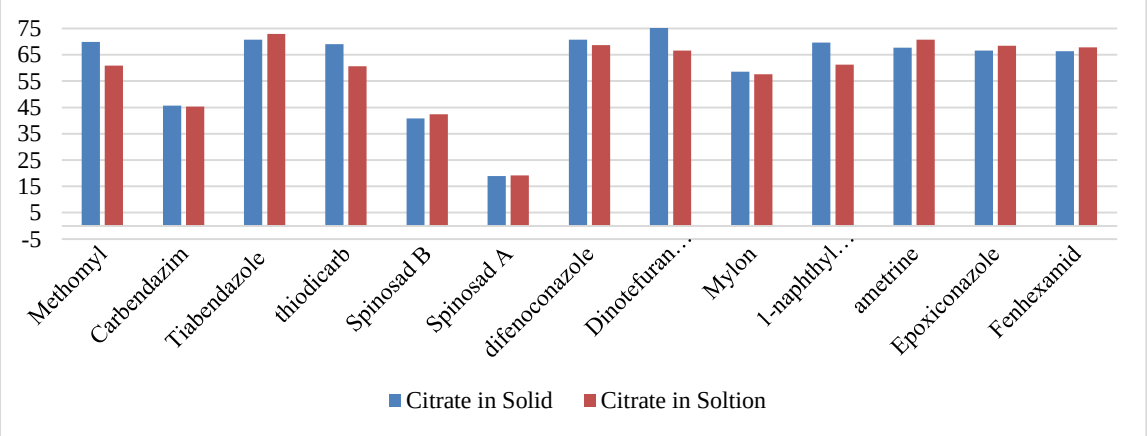

Figure 2: Comparison of the mode of addition of citrate salts for pH control in pesticide extraction in honey

Figures 1 and 2 show that some compounds (eg thiabendazole and imazalil), presented better coefficients of variation with the QuEChERS method, which is attributed to the presence of GCB in the modified method, since it is well known that this adsorbent affects recovery of these compounds. Similarly, it is observed that for some compounds the accuracy is the same in both methods (eg cimoxanil, dinotefuran, pyraclostrobin, among others) which indicates that, possibly, they are not significantly affected by the factors mentioned above. .

The Levene test (30) was applied again in order to assess whether there are significant differences between the accuracies (i.e. coefficients of variation) of the methods. The results for compounds with significantly different accuracies ($p < 0.05$) are reported in Figure 3.

Figure 3. Percentages of average recovery (% R), number of compounds with% R less than 70% and with coefficients of variation (% CV) greater than 15% for the different pH values evaluated

By relating the results shown in Figure 3 with those presented in Figures 1 and 2, it is found that for most cases, the accuracy obtained with the modified method is better than that of the original method. However, in some cases (e.g. pyrimethanil, dazomet and epoxiconazole), better accuracies are obtained with the QuEChERS method; although it is noteworthy that the precision of the modified method meets the precision criteria (28).

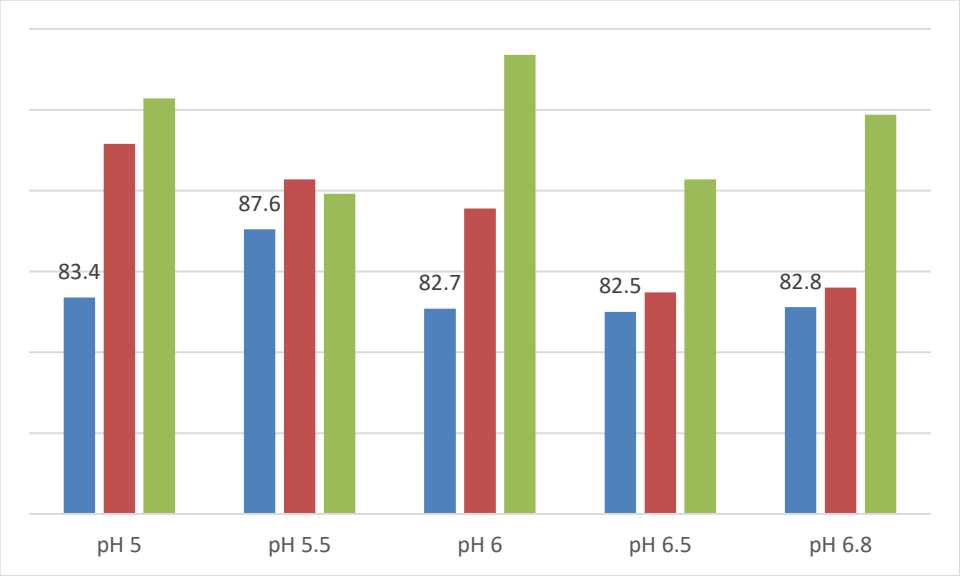

Figure 3. Percentages of average recovery (% R), number of compounds with% R less than 70% and with coefficients of variation (% CV) greater than 15% for the different pH values evaluated

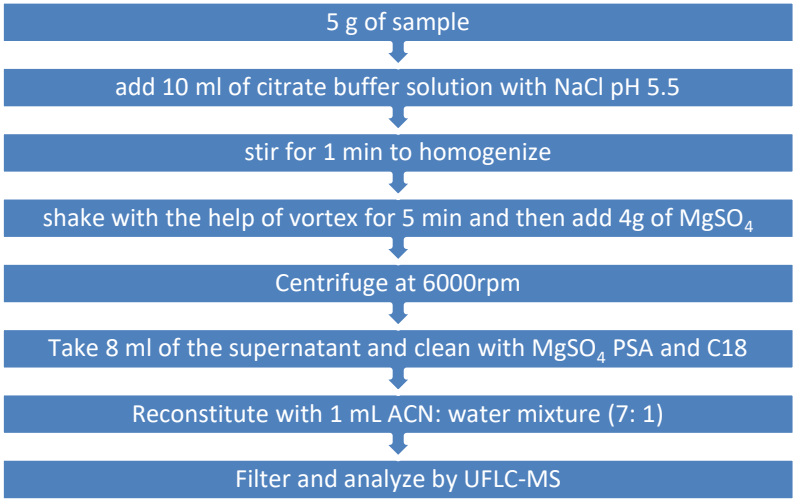

Figure 4. General scheme of the method developed for the analysis of pesticide residues in honey through UFLC-MS

Method recovery evaluation

A factorial experimental design was carried out that sought to determine if there were significant differences in the percentages of recovery of each of the methods, for this, a more real scenario was recreated using three types of pollen. Figures 4 and 5 show the recovery percentages obtained by each of the methods, for a single type of pollen.

Figures 4 and 5 show that the recovery percentages of most of the compounds are similar to each other and are within the acceptance range posed by the European Union (28). However, the recovery of some analytes by the two methods (e.g. pyraclostrobin, Benomyl and spinosad D) is very low, which suggests that pollen prevents their extraction; because unlike other matrices (e.g. fruits), an adequate recovery of the analytes is not possible using the QuEChERS method (11, 13). In this sense, the low recovery of these compounds is attributed to the matrix, since it is possible that there is an irreversible adsorption process or some type of reaction that prevents its determination is carried out, especially with chemical structures as complex as that of espinosad D.

The modifications that were made to the QuEChERS method facilitated the determination of several compounds such as thiodicarb and the group of neonicotinoids, because through the modified method it is possible to obtain acceptable recovery percentages (between 70% and 120%) that do not they can be achieved with the unmodified QuEChERS method.

In Figures 4 and 5 it can be seen that only seven compounds have better recoveries by the unmodified QuEChERS method, the remaining compounds have a better recovery by the modified method. The above implies that possibly the stages of sonication, cleaning or perhaps the longest time in the extraction process affect the stability of these seven compounds. However, when looking at Figures 1 and 2, it is found that for these same seven compounds there are better coefficients of variation, which indicates that in these cases the modified method, although it is of less truth, has a better accuracy (taking into account the current definition of this term.

Finally, Table 4 shows the probabilities of accepting each of the null hypotheses set out below:

H01: There is a significant interaction between the methods and the type of pollen.

H02: There are significant differences between the percentages of recovery of the methods.

H03: There are significant differences between the percentages of recovery in the different types of pollen.

The results in Table 4 show that only for Benomyl and spinosad D is rejected H01 ($p < 0.05$), which indicates that the recovery of these compounds depends on both the type of pollen and the method used. On the other hand, probabilities for H02, lower than 0.05, indicate that the null hypothesis is rejected and it is concluded that the recovery is statistically different between the two methods evaluated. By observing these results in detail and comparing them with Figures 4 and 5, it can be concluded that the recovery of the modified method is better ($p < 0.05$) for compounds such as acetate, thiabendazole, cymoxanil, azoxystrobin, hexaconazole, neonicotinoids, among others, since their recovery percentages are higher (see Figures 4 and 5) and are statistically different ($p < 0.05$). Likewise, it is found that only for tebuconazole there is a statistically better recovery percentage ($p < 0.05$), in the case of the unmodified method.

The probabilities obtained for H03 indicate that for the compounds that presented values below 0.05, there are differences in the recovery within the different types of pollen. However, when performing a Tukey test it was found that only for dimethoate there is a statistically different recovery for the modified method; The remaining compounds differences were given in the QuEChERS method. This implies that the modified method has better recovery percentages and these do not vary significantly ($\mu = 0.05$) when using other types of pollen.

Table 3. Results obtained in the validation of the method developed. Compound group 1- Acquisition method 1

Compuesto	Lower fortification concentration (µg / kg)	% Recovery to LC	% Recovery at 5 x LC	% Method recovery *	% CV maximum in intermediate precision conditions	LD (µg/kg)
Acephate	13.17	85.1	87.3	86.2	15.52	4.93
Monocrotophos	9.96	91.6	88.0	80 103.8	10.41	4.24
Oxamyl	9.78	103.2	90.5	80.4 1	11.58	4.12
Metomil	10.07	87.2	78.6	82.9	6.48	2.81
Benomyl	8.21	49.8	47.1	18.8 70.1	17.55	ND
Imidacloprid	12.55	94.5	84.2	72.3 97.9	12.28	6.12

Dimetoato	11.34	98.7	88.0	85 105.2	7.36	3.19
tiabendazole	10.70	97.1	83.2	90.2	8.09	1.36
cymoxanil	79.75	92.5	88.4	90.4	11.29	23.58
thiodicarb	8.92	97.3	95.9	96.6	9.62	2.51
Carbofuran	8.32	86.5	83.2	84.9	16.18	2.23
atrazine	5.87	93.2	83.0	72.8 94.9	14.02	2.05
metalaxyl	5.95	93.9	91.0	92.5	11.84	1.19
Pirimicarb	5.94	99.0	96.9	98	10.16	1.15
imazalil	8.21	94.0	83.7	73.8 100.7	6.95	2.19
3,4 -DPA	13.13	100.9	102.3	101.6	11.60	5.66
pyrimethanil	8.70	91.0	78.5	84.8	11.43	3.83
dimethomorph	6.01	102.1	104.5	103.3	10.08	1.63
azoxystrobin	14.69	51.7	49.6	32.4 58.5	6.52	ND
tebuconazole	15.95	93.5	93.7	81.9 102.5	12.80	1.98
methoxyfenozide	13.24	100.6	100.1	93.2 104.5	8.54	2.51
hexaconazole	10.93	95.0	99.3	87.1 108.4	13.03	2.85
spinosad a	14.69	37.1	39.7	38.4	6.91	ND
benalaxyl-m	88.36	90.5	88.6	75.4 102.4	7.80	14.63
difenoconazole	6.47	97.1	97.3	97.2	10.86	1.37
azinphos methyl	198.94	95.0	87.5	72.8 98	13.62	61.20
spinosad d	6.99	281.1	411.9	80.9 1033.4	81.99	ND
indoxacarb	28.75	91.9	95.7	82.1 99	8.36	6.15

* Estimated based on intermediate precision results. For the cases in which there was heterocedasticity between the different concentrations evaluated. the range of recovery percentages is presented.

ND: Not determined for this compound.

Table 4. Results obtained in the validation of the method developed. Compound group 2- Acquisition method 2

Compuesto	Lower fortification concentration (µg / kg)	% Recovery to LC	% Recovery at 5 x LC	% Method recovery *	% CV maximum in intermediate precision conditions	LD (µg/kg)
Metamidofos	41.70	88.9	96.5	92.7	13.85	6.15
Propamocarb	12.48	92.4	103.3	97.9	15.53	4.20
Dinotefuran	10.99	89.5	88.7	80 -106.6	15.09	3.17
Linuron	18.96	91.8	85.1	73.6- 99.5	15.12	7.20
Nitenpiram	13.46	85.6	102.9	94.3	12.94	2.95
Thiametoxan	16.67	97.3	97.3	97.3	4.43	4.75

Dazomet	52.03	84.4	83.9	70.1 1	14.85	18.02
Mevinphos	16.97	92.5	87.8	73.5 1	11.64	7.77
Imidacloprid	169.95	93.8	105.8	99.8	16.54	57.03
Acetamiprid	66.45	102.9	95.9	88.7 -105.8	12.12	11.13
Tiaclorprid	16.71	111.0	110.4	110.7	9.15	2.65
Simazina	5.87	98.5	97.3	97.9	12.02	0.85
Carbaryl	38.95	96.2	103.9	87.6 -112.9	9.32	7.13
Isoprocarb	44.11	93.7	86.2	90	14.95	9.53
Ametrina	13.26	91.6	98.1	94.9	9.35	1.36
3.4 dicloranilida	9.03	92.6	104.7	77.5 -115.8	10.35	0.95
Tridemoprh	74.91	99.7	79.9	74.5 -101.2	19.46	17.17
Epoxiconazole	85.22	97.4	105.8	81.1- 119.1	14.30	24.26
Fenexhamid	41.48	78.4	94.9	86.7	13.20	9.32
Etoprofos	30.08	86.8	91.3	89.1	12.28	8.70
Flusilazole	14.88	99.7	111.1	96.8- 115.3	7.52	4.92
Penconazol	12.42	94.6	110.5	102.6	11.09	2.41
Piraclistrobin	11.31	38.6	21.9	12.9 -68.6	21.14	ND
Trifloxystribina	8.46	89.3	102.8	81.3 -113.5	10.27	1.46
Lufenuron	8.57	31.5	32.7	32.1	34.59	ND
Benfuracarb	7.76	100.9	102.2	84.1 -111.9	13.77	2.28
Temefos	65.46	88.8	94.7	75.2 -112.8	16.98	29.00
Buprofezin	11.44	79.2	94.0	86.6	14.30	4.71

* Estimated based on intermediate precision results. For the cases in which there was heterocedasticity between the different concentrations evaluated. the range of recovery percentages is presented.
ND: Not determined for this compound.

Table 5. Linear interval of the analytical method

Compound	Lineal interval (ng / mL)	Compound	Lineal interval (ng / mL)
Acephate	18.8 -188.2	methamidophos	59.6 -595.7
Monocrotophos	14.2 -142.3	propamocarb	17.8- 178.2
Oxamyl	4 13-9.7	Thiocyclam	15.7- 57
Metomil	14.4- 143.9	Linuron	27.1- 270.8
Benomyl	11.7 -117.2	nitenpyram	19.2 -192.2
Imidacloprid	17.9 -179.3	thiamethoxam	23.8 -238.1
Dimetoato	16.2 -161.9	dazomet	74.3 -743.2
tiabendazole	15.3 -152.9	mevinphos	24.2- 242.4
cymoxanil	113.9- 1139.3	3-oh carbofuran	242.8- 2427.8
thiodicarb	12.7 -127.4	acetamiprid	94.9- 949.3
Carbofuran	11.9- 118.8	thiacloprid	23.9- 238.8
atrazine	8.4- 83.9	dinotefuran	8.4 -83.8

metalaxyl	8.5- 84.9	somazina	55.6 -556.4
Pirimicarb	8.5- 84.9	carbaryl	63 -630.1
imazalil	11.7- 117.2	isoprocarb	18.9- 189.5
3,4 -DPA	18.8 -187.6	Ametrina Proficol	12.9- 29
pyrimethanil	12.4 -124.3	tridemorph	07- 1070.1
dimethomorph	8.6 -85.8	epoxiconazole	121.7 -1217.5
azoxystrobin	2 20-9.8	fenhexamid	59.3- 592.6
tebuconazole	22.8 -227.8	ethoprophos	43- 429.7
methoxyfenozide	18.9 -189.2	flusilazole	21.3 -212.6
hexaconazole	15.6 -156.1	penconazole	17.7- 177.4
spinosad a	2 20-9.8	pyraclostrobin	16.2- 161.5
benalaxyl-m	126.2 -1262.2	trifloxystrobin	12.1- 120.8
difenoconazole	9.2 -92.4	lufenuron	12.2- 122.4
azinphos methyl	284.2 -2841.9	buprofezin	11.1- 110.9
spinosad d	0 9-9.8	clorfenamina	93.5- 935.1
indoxacarb	41.1 -410.7	temefos	16.3 -163.4

Conclusions

In this study it was found that the modified QuEChERS method, in general, has better detection limits, better recovery rates, better selectivity and better accuracy. On the other hand, it was shown that for 16 of the 58 pesticides in the present study, better recovery percentages were presented using the modified method and only for tebuconazole, a lower recovery percentage was presented, although not less than 70%. Finally, it was found that only for dimethoate, carbendazin and spinosad D the recovery percentages depend on the type of pollen in the modified method and only for three compounds recovery percentages below 70% were found.

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